APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1985, p. 244–246
0099-2240/85/010244-03$02.00/0

Vol. 49, No. 1

Hybridization and Polyploidization of *Saccharomyces cerevisiae* Strains by Transformation-Associated Cell Fusion

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Received 25 June 1984/Accepted 16 October 1984

Hybrid or polyploid clones of *Saccharomyces cerevisiae* produced by protoplast fusion were easily isolated by selecting transformants with the plasmid phenotype because the transformation was directly associated with cell fusion. When haploid cells were used as the original strain, the transformants were mostly diploids with a significant fraction of polyploids (triploids or tetraploids). Repeated transformation after curing the plasmid gave rise to clones with higher ploidy, but the frequency of cell fusion was severely reduced as ploidy increased.

Polyploids are often encountered among the strains of *Saccharomyces* used in the brewing (2, 3, 18), baking (4), and alcoholic fermentation industries (Y. Oshima, unpublished data). This suggests that polyploidy in *Saccharomyces* yeasts may provide some advantages in these industries. However, the biological significance of ploidy in yeasts has scarcely been investigated because of the lack of an efficient method to construct polyploid strains, especially an isogenic series of polyploid strains. Several methods for construction of heterogenic polyploid yeasts with the laboratory strains of *Saccharomyces cerevisiae* used for genetic study have been proposed. They include forced mating (12), respiration-deficient auxotroph mating (5), protoplast fusion (19), and the use of such mutations as *das* (17) and *hmra* (9, 11). These methods, however, are difficult to apply to industrial yeasts without suitable genetic markers for the selection of rare hybrid or polyploid cells. Another method, involving switching the mating type alleles by the function of the homothallic genes, has been suggested by Takano et al. (16). Takagi et al. (15) have applied this method and constructed various isogenic series of polyploid strains. However, this method is only applicable to sporogenic homothallic strains, and the polyploid strains constructed are still heterogenic for the mating type alleles.

Recently, we noted that transformation of protoplasted yeast cells is directly associated with cell fusion (7). Fused cells, which arise in low frequency (ca. 10^{-5} per protoplast), can be selected by picking up transformants with the phenotype of the plasmid marker, even when they arise from two cells of the same clone. This communication deals with the application of this finding to hybridization and polyploid construction in *S. cerevisiae*.

The protocol was to transform protoplasts with a plasmid, for example, a haploid strain, and to select transformants with the plasmid marker, most of which are fused diploid cells. Diploid or polyploid clones among the transformants can be distinguished under the microscope by their larger cell size than the original cells. When the diploid or polyploid transformants are cultivated under nonselective conditions for the plasmid marker, the plasmid is lost spontaneously from a fraction of cells during vegetative growth. These cured clones can be selected from cell population of the primary transformants and subjected to repeated transformation with the same plasmid to obtain higher polyploids. If transformants are isolated from protoplasts of single strain, they should be autodiploids or autopolyploids, whereas heterogenic diploid or polyploid clones can be isolated by mixing two kinds of protoplast, even if they lack complementary auxotrophic markers to select cell fusants.

To assess this method, haploid strains KM2C-43B (*MAT α ho HMLα HMRa sir2 trp1 his1 ade6*, obtained from A. J. S. Klar et al. [10]) and ATG3 (*MATa ho HMLα HMRa sir2 trp1 leu2-3,112 his1 his3-532*, constructed in our laboratory) were subjected to transformation under the conditions described previously (7) with a yeast vector marked with the *TRP1* gene of *S. cerevisiae*, YRp7 (14) or YCp19, whose gross structure was illustrated previously (7). These two haploid strains are marked with the *sir2* mutation, which allows expression of the cryptic mating type information at the *HML* and *HMR* loci as well as at the *MAT* locus (6, 10, 13). (For the other genetic symbols of *S. cerevisiae*, see Broach [1].) When these strains are autodiploidized, they should show the sporulation-competent phenotype (Spo^+) because their *HML* and *HMR* loci have respectively α and **a** information and they are expressed under the *sir2* mutation. We isolated 309 tryptophan-prototrophic (Trp^+) transformants of strain KM2C-43B with YRp7 and 485 Trp^+ transformants of strain ATG3 with YCp19. Since 259 of the 309 transformants of KM2C-43B with YRp7 and 230 of the 485 transformants of ATG3 with YCp19 showed the Spo^+ phenotype, these might be diploids. When cells of these Spo^+ clones were observed under the microscope, 225 of the 259 Spo^+ YRp7 transformants and 198 of the 230 Spo^+ YCp19 transformants showed diploid cell size, whereas the remaining 34 YRp7 transformants and 32 YCp19 transformants showed larger cell size than the diploid cells.

To confirm the ploidy of the Trp^+ Spo^+ transformants, some of them were sporulated and four-spored asci were dissected. Among the seven Trp^+ Spo^+ transformants of KM2C-43B with YRp7 and four of ATG3 with YCp19 examined, four of them, two from KM2C-43B and two of ATG3, were thought to be tetraploid because all of their spore segregants showed the Spo^+ phenotype in six to eight asci dissected for each transformant. The remaining seven Trp^+ Spo^+ transformants were thought to be diploid, because all of the spore segregants showed Spo^- phenotype with haploid cell size. Results of the cellular DNA determination agreed with the above judgments (Table 1).

This microscopic examination of transformants allowed detection of tetraploids in high frequency (approximately 13%, namely, 34 tetraploid among 259 transformants by YRp7 and 32 among 230 transformants by YCp19 in the

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TABLE 1. Characteristics of various isogenic series of haploid, diploid, and tetraploid clones constructed by transformation-associated cell fusion with plasmid YRp7 or YCp19[a]

Strain[b]	DNA contents[c] (mg/10^{11} cells)	Ratio[d]	Spo phenotype of tetrad clone[e]	Expected ploidy
KM2C-43B	2.9	0.9	No segregants	N
KM2C-43B-T7-C1	3.2	1.0	All Spo^-	2N
KM2C-43B-T78-C1	5.6	1.8	All Spo^+	4N
ATG3	2.7	0.6	No segregants	N
ATG3-T5-C1	4.8	1.0	All Spo^-	2N
ATG3-T38-C1	8.4	1.8	All Spo^+	4N
D13-1A	2.9	0.6	—[f]	N
D13-1A-T10-C1	4.6	1.0	—	2N
D13-1A-T10-C1-T35-C1	8.2	1.8	—	4N
DK-13D	2.7	0.5	—	N
DK-13D-T48-C1	5.3	1.0	—	2N
DK-13D-T58-C1	13.6	2.6	—	5N
NA87-11A	2.4	0.6	—	N
NA87-11A-T42-C1	4.3	1.0	—	2N
NA87-11A-T59-C1	7.7	1.8	—	4N
C1457-3A	1.9	0.5	—	N
C1457-3A-T15-C1	4.0	1.0	—	2N
C1457-3A-T109-C1	7.2	1.8	—	4N

[a] Transformation was performed under the conditions described in a previous communication (7) with YRp7 for all the strains, except that YCp19 was used for strain ATG3. Plasmid-cured clones appearing spontaneously in a fraction of the transformant cell population during the cultivation of the transformant on nutrient (per liter, yeast extract, 10 g; peptone, 20 g; adenine, 400 mg; glucose, 20 g) medium were isolated by replica plating onto the selective medium.

[b] The genotypes of KM2C-43B, ATG3, and D13-1A are described in the text. Those of DK-13D, NA87-11A, and C1457-3A are *MATα ho trp1 his3-532 leu2-3,112*, *MATα ho trp1 his3-532 leu2-3,112 pho3 pho5*, and *MATα ho trp1 leu2 his4 thr4 arg4*. These three strains were constructed in our laboratory.

[c] DNA was extracted from cells shaken at 30°C for 36 h in YPAD medium, and cellular DNA content was determined as described previously (15).

[d] Cellular DNA contents of the respective diploid clone was counted as 1.0.

[e] From 6 to 27 asci from the diploid or tetraploid clones that originated from KM2C-43B or ATG3 were dissected, and the tetrad segregants were tested for their sporulation phenotype (Spo^+/Spo^-) as described previously (15).

[f] —, Diploid or polyploid strains originated from strains other than KM2C-43B and ATG3 do no sporulate since they have the *SIR*$^+$ genotype.

TABLE 2. Effect of ploidy on the cell fusion frequency associated with transformation

Combination[a] (A + B)	No. of transformants tested[b]	No. of transformants showing phenotype of:			FNF value[c] (%)
		A	AB	B	
N + N	197	15	54	128	80
N + 2N	142	24	28	90	44
N + 4N	115	41	13	61	29
2N + N	280	20	51	209	42
2N + 2N	98	6	13	79	39
2N + 4N	86	20	5	61	15
4N + N	248	4	2	242	11
4N + 2N	115	6	0	109	—[d]
4N + 4N	126	4	0	122	—

[a] In the A + B cell fusion, isogenic series of haploid (N), diploid (2N), and tetraploid (4N) clones of strain D13-1A were used as A, and those of strain KM2C-43B were used as B.

[b] Transformation was performed with YRp7, and the Trp^+ clones were scored as the transformants.

[c] Frequency of nuclear fusion associated with transformation (FNF) was calculated by the method decribed previously (7).

[d] —, FNF value was not calculated since no hybrid class transformants were obtained.

single transformation of haploid protoplasts). This indicates that multiple cell fusion occurred frequently under the transformation conditions employed in this study.

To confirm the general applicability of this method, we tested various of the other strains used for yeast genetics and in every case easily obtained diploid and polyploid clones (Table 1). The cell morphologies of some of them are shown in Fig. 1. It should be noted that tetraploid strains constructed by this method may have a *MATa*/*MATa*/*MATa*/*MATa* or *MATα*/*MATα*/*MATα*/*MATα* configuration. Such types of tetraploid cells with a homogenic genetic background have rarely, if ever, been constructed before.

During the course of these experiments, it was noticed that the frequency of cell fusion associated with transformation appeared to decrease as ploidy increased. To confirm

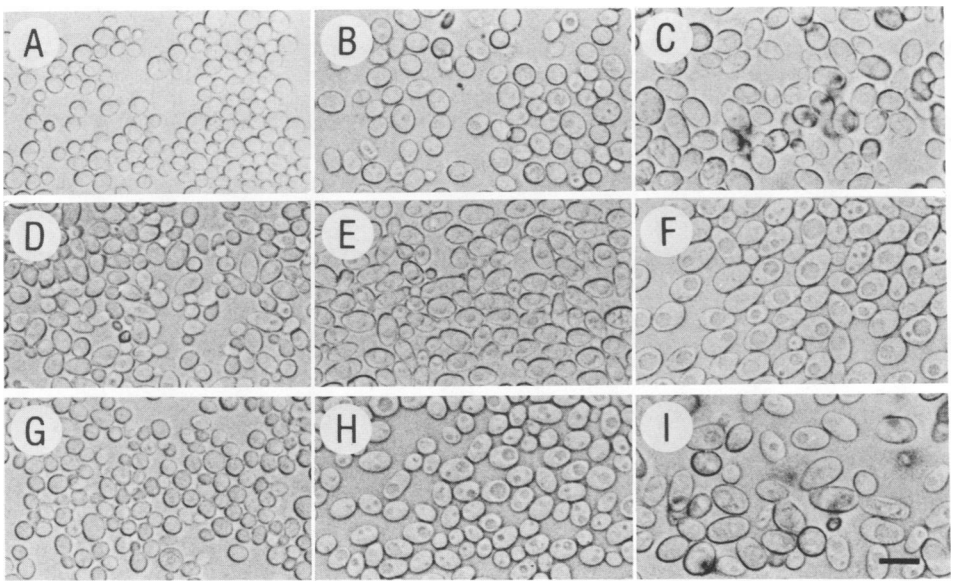

FIG. 1. Photomicrographs of yeast cells of isogenic series of strains D13-1A, KM2C-43B, and DK-13D. A, D13-1A (haploid); B, D13-1A-T10-C1 (diploid); C, D13-1A-T10-C1-T35-C1 (tetraploid); D, KM2C-43B (haploid); E, KM2C-43B-T7-C1 (diploid); F, KM2C-43B-T78-C1 (tetraploid); G, DK-13D (haploid); H, DK-13D-T48-C1 (diploid); I, DK-13D-T58-C1 (pentaploid). All photomicrographs were taken at the same magnification. Bar, 10 μm.

this, transformations were performed using various derivatives of two haploid strains, KM2C-43C (the genotype as described) and D13-1A (*MAT***a** *ho trp1 his3-532 gal2*, obtained from R. W. Davis Stanford University School of Medicine), and the fusion frequency was found to decrease significantly when the ploidy of the cells increased (Table 2). It was also difficult to construct octaploids by mating two tetraploid clones having the *MAT*α/*MAT*α/*MAT*α/*MAT*α and *MAT***a**/*MAT***a**/*MAT***a**/*MAT***a** genotype. We tried this method with tetraploid clones constructed by transformation-associated cell fusion from strains C1457-3A and D13-1A by mixing them in nutrient medium and selecting hybrid clones by the prototroph recovery method. However, the cell size of the several isolates examined was similar to that of tetraploid clones. This observation suggests that octaploid cells are extremely unstable and, even if they are transiently formed, readily degenerate to cells of lower ploidy during vegetative proliferation as suggested by Gunge and Nakatomi (5). In spite of this difficulty in obtaining polyploids higher than tetraploid, transformation-associated cell fusion was proven to be effective in construction of polyploid clones.

In this study, we employed plasmids having the *TRP1* gene as the plasmid marker. For such plasmid markers, the host strains should be auxotrophic with a *trp1* mutation. However, if plasmids bearing a dominant selective marker such as G418 resistance (8) are employed, this method should be applicable to any strain, including wild-type industrial strains.

This study was partially supported by grant-in-aid 57760087 for Encouragement of Young Scientists to S.H. from the Ministry of Education, Science, and Culture of Japan.

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